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Research

A dynamic model of UVR8 photoreceptor signalling in UV-B-acclimated *Arabidopsis*

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Summary

• The photoreceptor UVR8 mediates numerous photomorphogenic responses of plants to UV-B wavelengths by regulating transcription. Studies with purified UVR8 and seedlings not previously exposed to UV-B have generated a model for UVR8 action in which dimeric UVR8 rapidly monomerises in response to UV-B exposure to initiate signalling. However, the mechanism of UVR8 action in UV-B-acclimated plants growing under photoperiodic conditions, where UVR8 exists in a dimer/monomer photo-equilibrium, is poorly understood.

• We examined UVR8 dimer/monomer status, gene expression responses, amounts of key UVR8 signalling proteins and their interactions with UVR8 in UV-B-acclimated *Arabidopsis*.

• We show that in UV-B-acclimated plants UVR8 can mediate a response to a 15-fold increase in UV-B without any increase in abundance of UVR8 monomer. Following transfer to elevated UV-B, monomers show increased interaction with both COP1, to initiate signalling and RUP2, to maintain the photo-equilibrium when the dimer/monomer cycling rate increases. Native RUP1 is present in low abundance compared with RUP2.

• We present a model for UVR8 action in UV-B-acclimated plants growing in photoperiodic conditions that incorporates dimer and monomer photoreception, dimer/monomer cycling, abundance of native COP1 and RUP proteins, and interactions of the monomer population with COP1, RUP2 and potentially other proteins.

Introduction

UV-B wavelengths (280–315 nm) regulate numerous aspects of plant morphogenesis, physiology, biochemical composition and defence, principally by controlling the expression of hundreds of genes (Jenkins, 2009). Many responses to UV-B are mediated by the photoreceptor UV RESISTANCE LOCUS 8 (UVR8) (Jenkins, 2017; Yin & Ulm, 2017). Research in Arabidopsis has revealed that UVR8 regulates a wide range of processes, including metabolite biosynthesis, stem extension, leaf expansion, phototropism, photosynthetic competence, stomatal density, stomatal closure, circadian rhythmicity, flowering, and resistance to pathogens (Jenkins, 2017; Yin & Ulm, 2017). In addition, UVR8 is involved in responses to other stimuli, including osmotic stress (Fasano *et al.*, 2014) and UV-A light (Morales *et al.*, 2013), and inhibits thermomorphogenesis (Hayes *et al.*, 2017), and shade-avoidance responses (Hayes *et al.*, 2014). UVR8 is highly conserved in diverse plant taxa and is likely to be pivotal in mediating responses to UV-B in numerous species. It is therefore important to understand how UVR8 functions in plants growing in natural growth environments.

UVR8 is a seven-bladed β-propeller protein that exists as a dimer in the absence of UV-B (Rizzini *et al.*, 2011; Christie *et al.*, 2012; Wu *et al.*, 2012). UV-B absorption by specific UVR8 tryptophans causes dissociation of the dimer, enabling monomeric UVR8 to initiate signal transduction by direct interaction with other proteins. In particular, binding of CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1), associated with a SPA protein, to monomeric UVR8 sequesters COP1 from E3-ubiquitin-ligase complexes that degrade positive regulators of photomorphogenesis such as the ELONGATED HYPOCOTYL 5 (HY5) transcription factor (Favory *et al.*, 2009; Huang *et al.*, 2013). In consequence, HY5 accumulates following UV-B exposure and promotes transcription of many UVR8-regulated genes (Brown *et al.*, 2005; Brown & Jenkins, 2008; Favory *et al.*, 2009; Huang *et al.*, 2013). HY5 stimulates its own transcription (Binkert *et al.*, 2014), further increasing its accumulation. In addition, it was recently shown that specific transcription factors interact with UVR8. WRKY36 binds to the *HY5* promoter to repress transcription; direct interaction of WRKY36 with UVR8 in the nucleus following UV-B exposure relieves this repression, stimulating *HY5* expression (Yang *et al.*, 2018). The transcription factors BIM1 and BES1 mediate brassinosteroid (BR) signalling and stimulate extension growth; interaction with UVR8 in the nucleus reduces binding of these transcription

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factors to BR-responsive genes and hence promotes hypocotyl growth suppression by UV-B (Liang *et al.*, 2018). MYB73 and MYB77 regulate genes involved in auxin-controlled responses, several of which are inhibited by UV-B (Vanhaelewyn *et al.*, 2016; Jenkins, 2017); physical interaction of monomeric UVR8 with these transcription factors impairs their promoter binding activity and hence inhibits auxin-stimulated lateral root growth (Yang *et al.*, 2020).

UVR8 monomers are able to re-associate to form dimers (Christie *et al.*, 2012; Wu *et al.*, 2012; Heilmann & Jenkins, 2013; Heijde & Ulm, 2013). Whereas it takes many hours for the purified protein to re-dimerise ($t_{½}$ *c.* 24 h; Christie *et al.*, 2012; Wu *et al.*, 2012), the process occurs much more rapidly *in vivo* ($t_{½}$ *c.* 20 min; Heilmann & Jenkins, 2013) and is facilitated by interaction of UVR8 with REPRESSOR OF UV-B PHOTOMORPHOGENESIS 1 (RUP1) and RUP2 proteins (Heijde & Ulm, 2013). As the RUP proteins promote re-dimerisation, they constrain responses initiated by UVR8 monomer; hence Arabidopsis *rup1rup2* mutant plants exhibit enhanced responses to UV-B mediated by UVR8 (Grüber *et al.*, 2010). Expression of the *RUP* genes is stimulated by UV-B, mediated by UVR8, COP1 and HY5, and thus provides a negative feedback regulation of UVR8 activity (Grüber *et al.*, 2010). In addition, there is evidence that RUP proteins are components of an E3 ubiquitin ligase that targets HY5 for degradation and that COP1 antagonises the action of the RUPs by mediating their proteolysis (Ren *et al.*, 2019). We have shown previously that both COP1 and RUPs interact with a 27-amino acid region in the C-terminus of UVR8 (termed C27: amino acids 397–423; Cloix *et al.*, 2012). WRKY36, BIM1 and BES1 also interact with the C27 region (Liang *et al.*, 2018; Yang *et al.*, 2018). COP1 additionally interacts with the β-propeller core of the protein (Yin *et al.*, 2015). Binding of COP1 and MYB73/MYB77 to UVR8 is UV-B dependent, whereas that of the RUPs and other transcription factors is not (Cloix *et al.*, 2012; Yin *et al.*, 2015; Liang *et al.*, 2018; Yang *et al.*, 2018, 2020). It is proposed that the UVR8–COP1 interaction is disrupted during RUP-mediated re-dimerisation (Heijde & Ulm, 2013).

The above description of UVR8 action was developed both from studies with purified UVR8 protein and from *in vivo* experiments, principally using white-light-grown seedlings given their first exposure to UV-B, with responses monitored over minutes to several hours. While such studies have given valuable insights into UVR8 function, it is important to understand how the photoreceptor functions in mature plants in growth conditions that more closely resemble natural growth environments. We have found that UVR8 functions differently in plants growing in photoperiodic conditions with supplementary, low level UV-B (Findlay & Jenkins, 2016). In these UV-B-acclimated plants UVR8 is not exclusively present as a dimer during the dark period, and it does not entirely convert to a monomer when first exposed to UV-B during the light period. By contrast, UVR8 exists in a photo-equilibrium where *c.* 75% of the protein is in the dimeric form even in the presence of UV-B. This photo-equilibrium is dependent on the presence of RUP proteins, indicating that the rate of UV-B-induced dimer dissociation is countered by RUP-mediated re-dimerisation. Thus, in contrast to nonacclimated seedlings first exposed to light, UVR8 does not function as a simple dimer-to-monomer on/off switch in mature, light-grown, UV-B-acclimated plants.

Current models of UVR8 action are evidently inadequate to explain UVR8 function in plants growing under photoperiodic cycles in the presence of UV-B, which are the conditions plants generally experience in nature. Hence, the aim of the present study is to understand how UVR8 functions in light-grown, UV-B-acclimated plants. We examined the response of UV-B-acclimated plants to a substantially increased level of UV-B by monitoring UVR8 dimer/monomer status, changes in gene expression, amounts of COP1 and native RUP proteins and their interactions with UVR8. This research enabled us to develop a model for UVR8 action and regulation in UV-B-acclimated plants grown in photoperiodic conditions.

Materials and Methods

Plant material

All experiments were undertaken with the *Arabidopsis thaliana* (L*er*) *uvr8-1/CaMV35S$_{pro}$:GFP-UVR8* transgenic line described by Cloix & Jenkins (2008). The level of GFP-UVR8 expression is shown in Supporting Information Fig. S1. Plants were grown on agar plates containing half-strength Murashige and Skoog (½MS) salts in a growth cabinet at 20°C. Non-salts in a growth cabinet at-acclimated plants were grown for 2 wk in a 16 h : 8 h, white light : dark photoperiod, with 120 μmol m^{-2} s^{-1} white light provided by warm white light-emitting diodes (LEDs). UV-B-acclimated plants were grown under the same conditions, except that the white light was supplemented with 0.2 μmol m^{-2} s^{-1} UV-B from a broadband source (UVB-313 tubes; Q-Lab, Westlake, OH, USA; Cloix *et al.*, 2012). For treatment with elevated UV-B, plants were exposed to 3 μmol m^{-2} s^{-1} UV-B from the same source for up to 3 h, starting 3 h into the light period.

Immunodetection of proteins

Protein extraction from tissue samples, SDS-PAGE and immunodetection were carried out essentially as described previously (Kaiserli & Jenkins, 2007). Ponceau-stained RuBisCO large subunit (rbcL) was used as a loading control. CHS protein was detected as described previously (Heilmann *et al.*, 2016) using an antibody from Santa Cruz Biotechnology (Heidelberg, Germany). Production of the COP1 antibody was reported by Lian *et al.*, (2011).

Peptide antigens were synthesised to produce polyclonal antibodies specific to RUP1 (GALEIFSGKQS) and RUP2 (NTLHPHKQQQEQA). The antibodies were produced in rabbits and affinity purified by Cambridge Research Biochemicals (Cambridge, UK).

Representative blots are shown in the figures and additional blots are shown in Fig. S2.

UVR8 dimer/monomer status

The relative abundance of GFP-UVR8 dimer and monomer was assayed by immunodetection on Western blots following SDS-PAGE with nonboiled samples as described previously (Cloix *et al.*, 2012; Heilmann *et al.*, 2016). Immunoblots were incubated with an anti-GFP antibody (Clontech, Saint-Germain-en-Laye, France) and the relative abundance of bands was quantified as described by Findlay & Jenkins (2016).

Transcript assays

Transcript levels were assayed by qRT-PCR as described by Díaz-Ramos *et al.* (2018). Transcripts were quantified relative to control *ACTIN2* transcripts. The primers used were: *HY5* (5′-GCTGCAAGCTCTTTACCATC-3′ and 5′-AGCATCTGGTTCTCGTTCTG-3′); *RUP1* (5′-CGGTCGGGTTATCGGGTCAG-3′ and 5′-GAGCCATTGTAAAGCGTGTAGTCC-3′); *RUP2* (5′-TGAATTCGATCCCACTGATAACA-3′ and 5′-AGGGAGGCCGTAAAAACGA-3′); and *ACTIN2* (5′-CTTACAATTTCCCGCTCTGC-3′ and 5′-GTTGGGATGAACCAGAAGGA-3′).

Co-immunoprecipitation

Plants were grown on agar plates and exposed to UV-B as described above. Whole cell extracts were prepared as described by Kaiserli & Jenkins (2007). The co-immunoprecipitation assays were carried out using anti-GFP microbeads (μMacs, 130-091-125; Miltenyi Biotec, Bergisch Gladbach, Germany) to immunoprecipitate GFP-UVR8, and the presence of COP1 and RUP2 in the immunoprecipitates was examined essentially as described previously (Cloix *et al.*, 2012). The 'input' samples applied to the microbead columns and the immunoprecipitate eluates were analysed by SDS-PAGE followed by Western blotting and immunodetection using the anti-GFP, anti-COP1 and anti-RUP2 antibodies mentioned above.

Results

UV-B-acclimated plants can respond to elevated UV-B without any increase in UVR8 monomer

In this study plants were grown for 2 wk under photoperiodic conditions in white light supplemented with a very low fluence rate of UV-B (0.2 μmol m^{-2} s^{-1}). The constant presence of UV-B during the photoperiod was not detrimental to growth and the UV-B treated plants looked similarly healthy to plants growing without UV-B (Fig. 1a). Consistent with previous research (Jenkins, 2017; Yin & Ulm, 2017), the plants acclimated to the presence of UV-B through morphological and biochemical changes. The UV-B-acclimated plants had shorter petioles and appeared more compact. In addition, UV-B-acclimated plants expressed CHS protein (Fig. 1b), which facilitates the synthesis of UV-protective flavonoids that enable plants to tolerate exposure to elevated UV-B. Transfer of the UV-B-acclimated plants to a 15-fold higher fluence rate of UV-B (3 μmol m^{-2} s^{-1}) elicited only a moderate change in the level of CHS over a 3-h period (Fig. 1b lower panel). By contrast, plants not previously acclimated to UV-B showed a substantial increase in CHS protein over the same period (Fig. 1b upper panel), consistent with the induction of UV-protection.

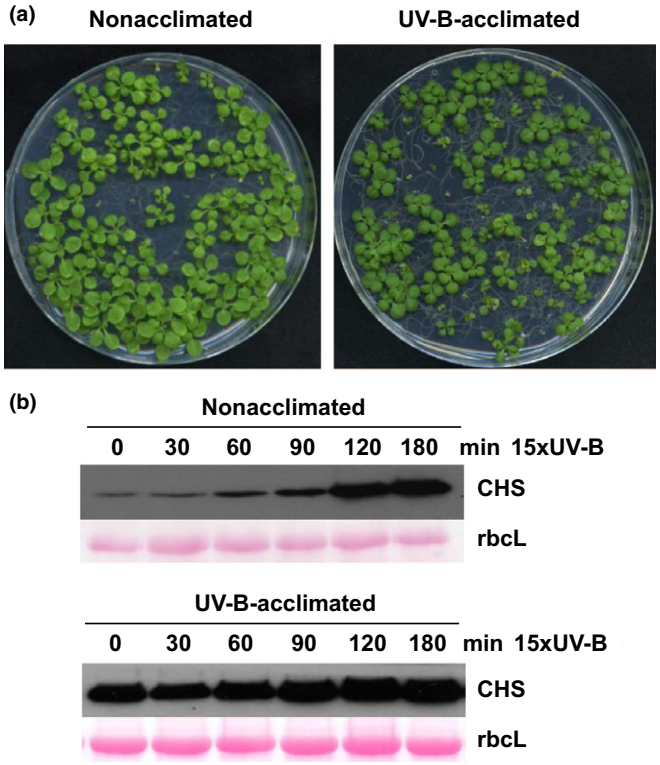

Fig. 1 *Arabidopsis thaliana* plants acclimate to a very low fluence rate of UV-B. (a) Appearance of UV-B-acclimated and nonacclimated plants. GFP-UVR8 plants were grown for 2 wk under a 16 h : 8 h, light : dark cycle, either without (nonacclimated) or with (UV-B-acclimated) supplementary 0.2 μmol m^{-2} s^{-1} UV-B. (b) CHS protein levels in UV-B-acclimated and nonacclimated plants. Nonacclimated or UV-B-acclimated plants grown as in (a) were exposed to 3 μmol m^{-2} s^{-1} UV-B (15×UV-B) for up to 3 h. CHS protein in extracts was immunodetected on Western blots using an anti-CHS antibody. Ponceau-stained rbcL is shown as a loading control.

In plants grown in white light minus UV-B, GFP-UVR8 was present as a dimer (Fig. 2a). When these nonacclimated plants were exposed to 3 μmol m^{-2} s^{-1} UV-B the dimer substantially converted to the monomer, consistent with previous findings (Rizzini *et al.*, 2011; O'Hara & Jenkins, 2012; Huang *et al.*, 2013). Plants grown for 2 wk under white light with supplementary 0.2 μmol m^{-2} s^{-1} UV-B established a UVR8 photo-equilibrium in which *c.* 30% of the protein was in the monomeric form (Fig. 2b,c) (Findlay & Jenkins, 2016). It is important to note that UV-B-acclimated plants did not significantly increase the level of monomer on being exposed to a 15-fold higher fluence rate of UV-B, in contrast with the nonacclimated plants (Fig. 2c).

We examined whether plants grown as above initiated transcript-level responses when transferred to the elevated fluence rate of UV-B. UV-B-acclimated plants had two-fold to three-fold

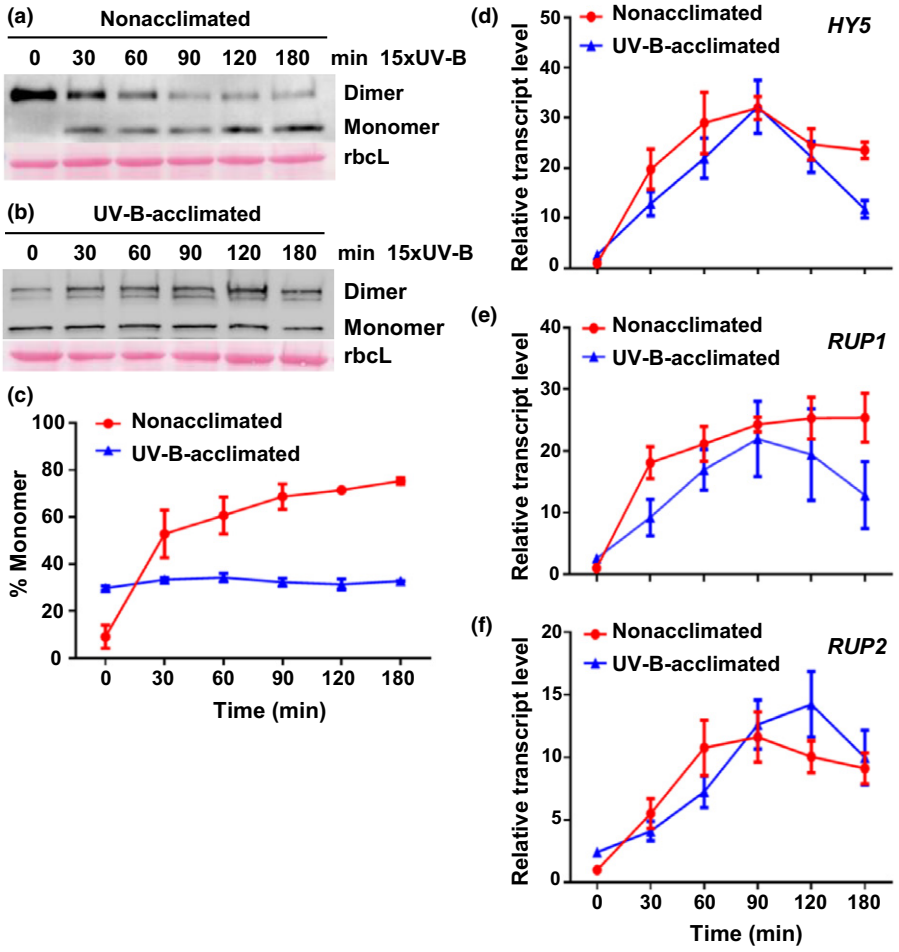

Fig. 2 UVR8 dimer/monomer status and gene expression response in UV-B-acclimated and nonacclimated *Arabidopsis thaliana* plants. Plants grown for 2 wk in 16 h : 8 h, white light : dark cycle either without (nonacclimated) or with (UV-B-acclimated) supplementary 0.2 μmol m^{-2} s^{-1} UV-B were exposed to 3 μmol m^{-2} s^{-1} UV-B (15×UV-B) for up to 3 h. (a, b) Dimer/monomer status of GFP-UVR8 was assayed by SDS-PAGE with nonboiled samples and immunodetection with an anti-GFP antibody. Ponceau-stained rbcL is shown as a loading control. (c) Quantification of % monomer/total UVR8; mean ± SE of three biological replicates. (d–f) Gene expression in UV-B-acclimated and nonacclimated plants. Plants were grown and exposed as above. Levels of (d) *HY5*, (e) *RUP1* and (f) *RUP2* transcripts relative to *ACTIN2* control were quantified by qRT-PCR; data shown are the mean ± SE of three biological replicates.

higher levels of the transcripts examined compared with nonacclimated plants (Fig. 2d–f), although this was dwarfed by the large increases observed when both types of plants were transferred to the 15-fold higher UV-B fluence rate. Both the nonacclimated and UV-B-acclimated plants showed a very similar, large increase in *HY5* transcript level following transfer to 3 μmol m^{-2} s^{-1} UV-B (Fig. 2d). Similar results were obtained for other transcripts, for example *RUP1* and *RUP2* transcripts (Fig. 2e,f). Evidently, in the UV-B-acclimated plants these large transcript-level responses to elevated UV-B occurred without a significant change in level of UVR8 monomer.

Increased UVR8 activity in UV-B-acclimated plants correlates with increased association of COP1 with UVR8

In nonacclimated seedlings, UV-B induces UVR8 monomerisation, and the monomers interact with COP1 to initiate responses (Rizzini *et al.*, 2011; Cloix *et al.*, 2012; Huang *et al.*, 2013). We used co-immunoprecipitation (Co-IP) assays to examine whether UVR8 monomers, which are constantly present in UV-B-acclimated plants, are bound to COP1 and whether transfer to elevated UV-B affects the interaction. Plants grown as above in the presence or absence of 0.2 μmol m^{-2} s^{-1} UV-B were exposed to 3 μmol m^{-2} s^{-1} UV-B and GFP-UVR8 was immunoprecipitated as described previously (Cloix *et al.*, 2012; Heilmann *et al.*, 2016). In nonacclimated plants, COP1 was not detectable in the immunoprecipitates (IPs) before UV-B exposure, but association of COP1 with GFP-UVR8 increased rapidly following transfer to elevated UV-B, as found in previous studies (Fig. 3a,b). UV-B-acclimated plants showed detectable COP1 interaction with GFP-UVR8, which increased following transfer to elevated UV-B, as in nonacclimated plants (Fig. 3a,b). The abundance of COP1 in plant protein extracts was assayed by immunodetection on Western blots (Fig. 3c). Quantification (Fig. 3b, left panel)

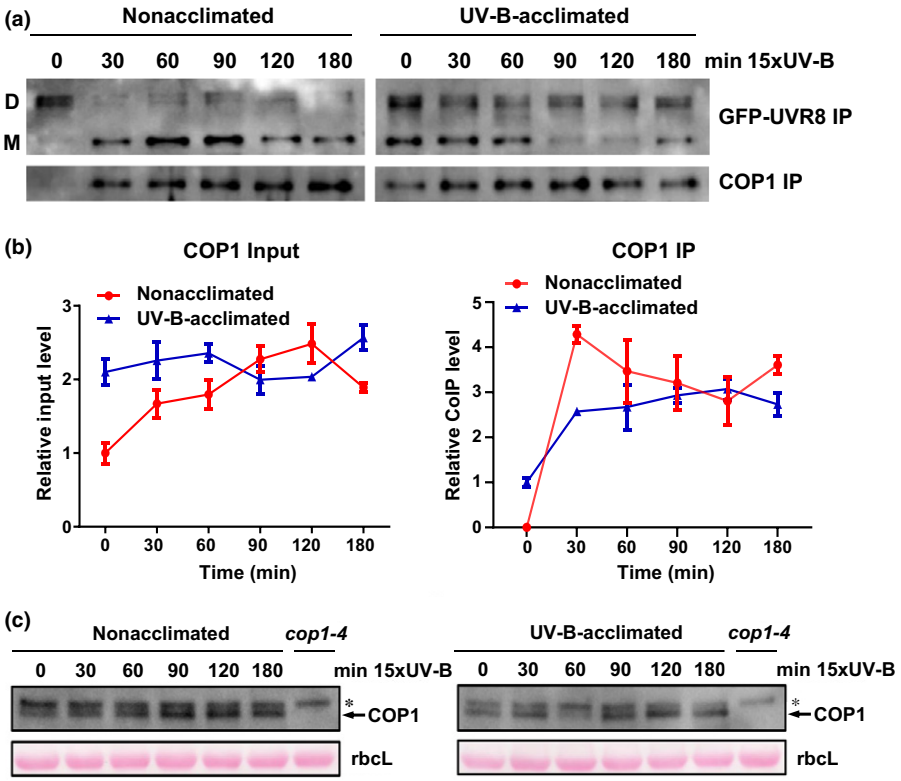

Fig. 3 Interaction of COP1 with UVR8 in UV-B-acclimated and nonacclimated *Arabidopsis thaliana* plants. Plants grown for 2 wk in 16 h : 8 h, white light : dark cycle either without (nonacclimated) or with (UV-B-acclimated) supplementary 0.2 μmol m^{-2} s^{-1} UV-B were exposed to 3 μmol m^{-2} s^{-1} UV-B (15×UV-B) for up to 3 h. (a) GFP-UVR8 was immunoprecipitated from protein extracts and samples run on SDS-PAGE without boiling to resolve dimer (D) and monomer (M). Levels of COP1 in the immunoprecipitates (IP) were determined using an anti-COP1 antibody. (b) Left panel: quantification of COP1 in the Input samples used for immunoprecipitation relative to a rbcL loading control. Right panel:quantification of COP1 abundance relative to GFP-UVR8 in the IPs normalised to differences in COP1 abundance in the Input samples; data shown are the mean ± SE of three biological replicates. (c) COP1 in extracts was immunodetected on Western blots using the corresponding antibody. Ponceau-stained rbcL is shown as a loading control. *cop1–4* mutant plants are shown as controls. *, nonspecific band.

showed a moderate increase in COP1 abundance in nonacclimated plants following UV-B exposure, but there was little, if any, change in COP1 abundance following transfer of UV-B-acclimated plants to elevated UV-B (Fig. 3b,c). Relative levels of interaction between COP1 and GFP-UVR8 observed in the Co-IP assays were normalised to differences in the abundance of COP1 (Fig. 3b). The increased interaction between UVR8 and COP1 seen when both types of plants were exposed to the elevated level of UV-B correlates with the observed increases in gene expression (Fig. 2).

UV-B exposure strongly increases accumulation of RUP2 in nonacclimated plants, but not in UV-B-acclimated plants

To facilitate understanding of the dynamics of UVR8 signalling, we assayed levels of native RUP proteins. To do this we produced antibodies against peptides of RUP1 and RUP2 (Fig. S3a). The specificity and effectiveness of these antibodies in detecting the corresponding RUP proteins was demonstrated using Western blots of the proteins expressed in yeast cells (Fig. S3b). RUP2 was readily detectable in 10-d-old plants exposed to UV-B (Fig. 4a), whereas RUP1 was usually below the limit of detection (Fig. 4b). RUP1 was detectable in seedlings grown with supplementary UV-B, but at very low levels (Fig. 4c). The observation that *RUP1* transcripts are readily detectable in plants exposed to UV-B (Fig. 2e) suggests that RUP1 may be an unstable protein.

RUP2 protein accumulated strongly when nonacclimated plants were exposed to UV-B (Fig. 5a; quantified in Fig. 5c Input panel), consistent with the increase in *RUP2* transcripts (Fig. 2f). However, in UV-B-acclimated plants, there was no apparent increase in RUP2 following exposure to elevated UV-B (Fig. 5a,c).

Exposure of plants to elevated UV-B increases interaction of RUP2 with UVR8

The interaction of RUP proteins with UVR8 was examined by Co-IP assays. RUP2 interaction was barely detectable under minus UV-B conditions in nonacclimated plants and the amount of co-immunoprecipitated protein increased following UV-B exposure (Fig. 5b,c). RUP2 interaction with UVR8 was also at a very low level in UV-B-acclimated plants and increased under elevated UV-B, as in nonacclimated plants (Fig. 5b,c). RUP1 was below the limit of detection in the IPs in these experiments.

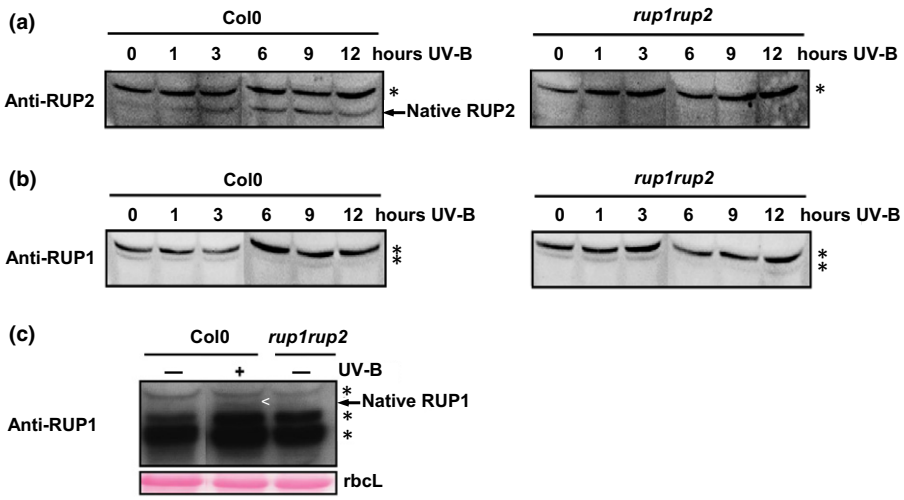

Fig. 4 Native RUP2 is more abundant than RUP1 in *Arabidopsis thaliana*. (a, b) 10-d-old Col0 and *rup1rup2* plants were grown under constant white light and then exposed to 3 μmol m^{-2} s^{-1} narrowband UV-B for the indicated times. Western blots of protein extracts were incubated with antibodies specific to RUP2 (a) or RUP1 (b). *, nonspecific bands. (c) Seedlings were grown under 2 μmol m^{-2} s^{-1} constant white light supplemented with (+) or without (−) 1.5 μmol m^{-2} s^{-1} UV-B for 4 d. A Western blot of protein extracts was incubated with anti-RUP1 antibody. The arrow indicates RUP1. *, nonspecific bands.

Discussion

Here we develop a model to explain how UVR8 behaves in UV-B-acclimated plants growing under photoperiodic conditions. There are clear differences to how it behaves in nonacclimated seedlings when they are first exposed to UV-B. Understanding how UVR8 functions in photoperiodically grown, UV-B-acclimated plants is important because UV-B regulates diverse responses that modulate metabolism and development and enhance viability.

UVR8 signalling is dependent on the activity, not amount, of UVR8 monomer

In a previous study with UV-B-acclimated plants growing under photoperiodic conditions, we found that a UVR8 photoequilibrium of *c.* 75% dimer/25% monomer was maintained regardless of a 10-fold difference in UV-B fluence rate (Findlay & Jenkins, 2016). We hypothesised that maintaining a photoequilibrium with a substantial pool of dimer might enable plants to respond effectively to a sudden, large increase in ambient UV-B by rapidly forming more monomer to initiate protective responses. However, our present observations did not support this hypothesis. No significant increase in monomer/total UVR8 was observed when UV-B-acclimated plants were exposed to a 15-fold higher UV-B fluence rate. Nevertheless, the plants responded with a large increase in expression of *HY5* and other genes, similar to nonacclimated plants. While some increase in UVR8 monomer may be observed under particular experimental conditions, for example when plants in shaded environments are exposed to sun flecks (Moriconi *et al.*, 2018), it is evident that an increase in monomer is not required to mediate a substantial response to elevated UV-B. Clearly, increased dimer dissociation is not necessary to mediate a response to UV-B, although the presence of monomer is essential. Consistent with this interpretation, we previously reported that plants expressing a constitutively monomeric, mutant UVR8 protein have similar UV-B responses to wild-type, demonstrating that both the ability to form a dimer and dimer dissociation are dispensable (Heilmann *et al.*, 2016).

The research raises the question of what, if any, benefit there is in plants possessing the UVR8 dimer. One possibility is that the dimer provides a mechanism for modulating the level of potentially active monomer under certain conditions, either to generate more monomer to enhance responses, or to reduce it to constrain UVR8 signalling. In addition, regulating the relative abundance of dimer/monomer provides a mechanism for modulating sequestration, and hence activity, of proteins that interact specifically with the monomer, such as COP1 and several transcription factors. Another possibility is that monomer-to-dimer conversion provides a mechanism for non-UV-B signalling pathways to regulate UVR8 signalling; evidence was presented previously that temperature can influence the rate of dimerisation (Findlay & Jenkins, 2016).

Increased UV-B exposure stimulates dimer/monomer cycling

It is important to consider how a substantial increase in UV-B response in UV-B-acclimated plants could occur without any significant change in the amount of monomer. One possibility is that the rate of dimer dissociation, and hence monomer formation (number of monomers formed per unit time), determines the level of response. The rate of monomerisation will increase in proportion to the increase in UV-B fluence rate, as shown in previous dose–response analyses (Díaz-Ramos *et al.*, 2018). However, as the steady-state monomer/total UVR8 fraction remains the same, there must be a corresponding increase in the rate of

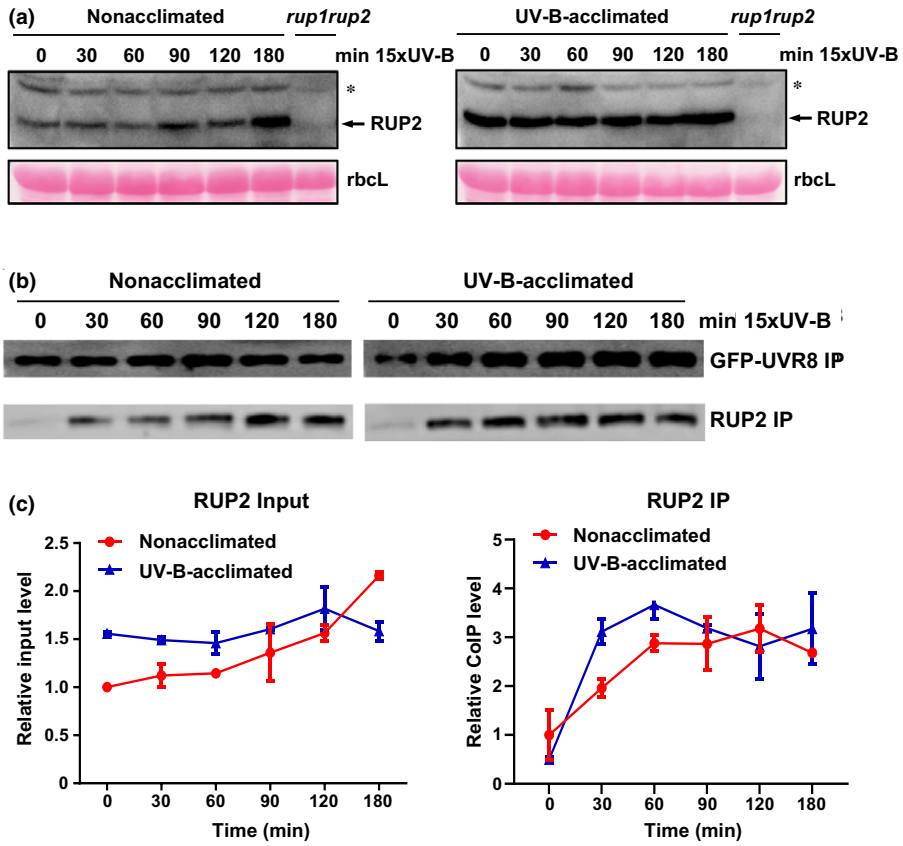

Fig. 5 Interaction of RUP2 with UVR8 in UV-B-acclimated and nonacclimated *Arabidopsis thaliana* plants. Plants grown for 2 wk in 16 h : 8 h, white light : dark cycle either without (nonacclimated) or with (UV-B-acclimated) supplementary 0.2 μmol m^{-2} s^{-1} UV-B were exposed to 3 μmol m^{-2} s^{-1} UV-B (15×UV-B) for up to 3 h. (a) Native RUP2 in protein extracts was immunodetected on Western blots using the corresponding antibody. Ponceau-stained rbcL is shown as a loading control. *rup1rup2* mutant plants are shown as controls. *Indicates a nonspecific band. (b) GFP-UVR8 was immunoprecipitated from protein extracts and samples run on SDS-PAGE. Levels of RUP2 in the immunoprecipitates (IP) were determined using an anti-RUP2 antibody. (c) Left panel: quantification of RUP2 in the Input samples used for immunoprecipitation relative to a rbcL loading control. Right panel: quantification of RUP2 abundance relative to GFP-UVR8 in the IPs normalised to differences in RUP2 abundance in the input samples; data shown are the mean ± SE of three biological replicates.

re-dimerisation, mediated by RUP proteins. It is therefore evident that increased UV-B exposure stimulates the rate of dimer/monomer cycling. It is important to consider whether the magnitude of UVR8-mediated response would be dependent on the actual rate of monomer formation, or on the steady-state amount of monomer. As interaction of the monomer with COP1 or specific transcription factors is critical in initiating a response, the amount of monomer available for interaction is likely to be more important than the rate of its formation. There are interesting parallels here with the potential role of the Pr-Pfr cycling rate in phytochrome action, whereas the cycling rate will increase with fluence rate, the extent of response is determined by the amount of active Pfr (Mancinelli, 1994).

UVR8 monomer photoreception likely contributes to UV-B perception in UV-B-acclimated plants

It is likely that photoreception by UVR8 monomers is at least partially responsible for the observed increase in gene expression in UV-B-acclimated plants. As mentioned above, photoreception by monomeric UVR8 efficiently mediates UV-B responses (Heilmann *et al.*, 2016), and monomer photoreception would permit an increase in response without an increase in monomer abundance, consistent with the present findings. Approximately 30% of UVR8 is in the monomeric form in UV-B-acclimated plants (Fig. 2c). When plants are transferred to elevated UV-B, rates of both dimer and monomer photoreception should increase in proportion to the increase in fluence rate, and both types of photoreception are likely to contribute to an increase in levels of signalling-active monomer. It is proposed that photoreception 'activates' the monomer in some way so that it can interact with relevant proteins to initiate a response (Jenkins, 2014). Such activation could, for instance, involve observed conformational changes to the monomer (Heilmann *et al.*, 2014; Miyamori *et al.*, 2015; Zeng *et al.*, 2015; Camacho *et al.*, 2019). There is evidence that conformational changes to the monomer alter the exposure of the C-terminal region that interacts with other proteins (Camacho *et al.*, 2019). Current models of UVR8 signalling

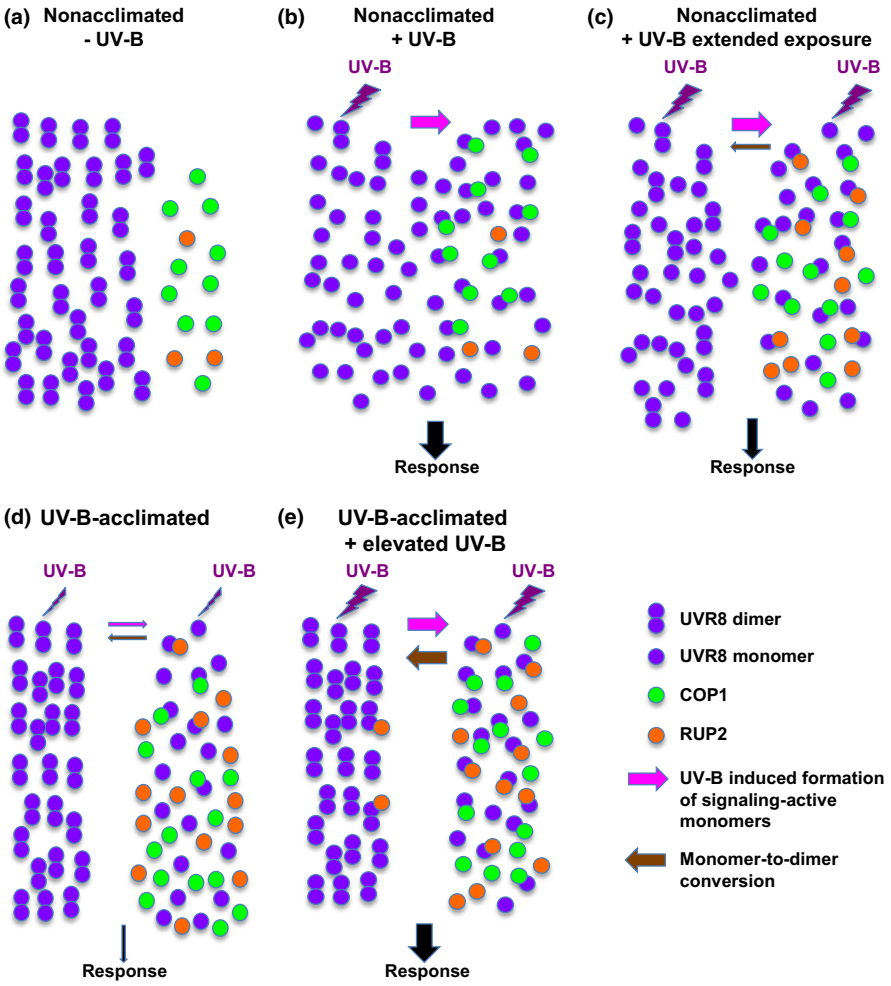

Fig. 6 Model of UVR8 action in *Arabidopsis thaliana*. In nonacclimated plants (a) UVR8 exists as a dimer. Photoreception by the dimer (b) produces signalling-active monomers that interact with COP1 to initiate transcriptional responses, including stimulation of *RUP2* expression. During extended exposure to UV-B (c) RUP2 binds to UVR8 monomers to promote re-dimerisation and the magnitude of response decreases. Continued exposure will lead to the acclimated state. In UV-B-acclimated plants (d) a dimer–monomer photo-equilibrium is established with *c.* 30% of total UVR8 in the monomeric form. Some monomers bind COP1 to maintain a low level of response and some will bind to RUP2 to maintain the photo-equilibrium. Exposure of UV-B-acclimated plants to elevated UV-B (e) increases photoreception stimulating both COP1 binding to enhance the level of response and RUP2 binding to increase the rate of re-dimerisation to maintain the photo-equilibrium. In all conditions, monomers that are not bound to COP1 or RUP2 may interact with other proteins. Both dimers and monomers are shown as being active in photoreception.

involve the monomer binding to COP1, which results in stabilisation of HY5 protein (Favory *et al.*, 2009; Rizzini *et al.*, 2011; Huang *et al.*, 2013), which in turn can stimulate downstream responses including its own transcription (Binkert *et al.*, 2014). Signalling also involves direct interaction of UVR8 monomers with specific transcription factors to initiate downstream responses (Liang *et al.*, 2018; Yang *et al.*, 2018, 2020). However, there is no evidence that monomer photoreception is more, or less, likely to initiate signalling than dimer photoreception.

UV-B activation increases the proportion of the monomer population associated with COP1

Previous studies have shown that COP1 interacts with monomeric UVR8 and not with the dimer (Rizzini *et al.*, 2011; Cloix *et al.*, 2012; Huang *et al.*, 2013; Yin *et al.*, 2015). Co-IP experiments (Fig. 3) indicate that increased UVR8 photoreception following transfer of UV-B-acclimated plants to elevated UV-B generates an increased level of activated monomers that interact with COP1. In UV-B-acclimated plants, a low level of COP1 association with UVR8 was detected before transfer to elevated UV-B. This interaction likely promotes the low level of gene expression required to maintain the UV-B-acclimated state (Fig. 2). The substantial increase in UVR8–COP1 interaction following transfer to elevated UV-B correlates with the large stimulation of gene expression. As the steady-state level of monomers did not change in the UV-B-acclimated plants, an increased proportion of the monomer population must have become activated as a result of dimer and/or monomer photoreception. Both dimer (Rizzini *et al.*, 2011) and monomer (Heilmann *et al.*, 2016) photoreception generate monomers able to bind to COP1.

Elevated UV-B increases binding of UVR8 monomer to RUP2 and a concomitant increase in dynamics of UVR8

The abundance of native RUP2 increased strongly following exposure of nonacclimated plants to UV-B. RUP2 accumulated in UV-B-acclimated plants and there was no change in abundance when these plants were transferred to elevated UV-B. By contrast, RUP1 was difficult to detect under all conditions examined, both in protein extracts and in Co-IP assays with UVR8. It is therefore likely that RUP2 is functionally more important than RUP1 in leaf tissue, consistent with the phenotypes of the corresponding *rup* mutants (Grüber *et al.*, 2010).

RUP2 showed a strong increase in interaction with UVR8 during UV-B exposure of nonacclimated plants. The initial increase occurred without any significant change in RUP2 abundance. The rapid increase in RUP2 accumulation and interaction with UVR8 will likely facilitate the establishment of the dimer/monomer photo-equilibrium. In UV-B-acclimated plants there was also a strong increase in RUP2–UVR8 interaction following exposure to elevated UV-B, which correlates with the increased rate of UVR8 dimer/monomer cycling. RUP2–UVR8 interaction likely facilitates re-dimerisation, maintaining the steady-state monomer/dimer photo-equilibrium when the rate of monomer formation increases at the 15-fold higher UV-B fluence rate. Clearly there is a sufficiently large capacity for re-dimerisation in UV-B-acclimated plants to maintain the constant level of monomer following exposure to elevated UV-B.

Model for UVR8 action in UV-B-acclimated plants

The Co-IP assays show that both RUP2 and COP1 increase in association with UVR8 following UV-B exposure of both nonacclimated and UV-B-acclimated plants. Both proteins bind to the same C27-amino acid region in the C-terminus of UVR8 (Cloix *et al.*, 2012) and therefore a single UVR8 monomer could only interact directly with either COP1 or RUP2 via this site at any one time. Hence, to interpret our findings we consider the UVR8 monomer population as a whole and the proportion of molecules binding either COP1 or RUP2. A new model for UVR8 action is shown in Fig. 6.

In nonacclimated plants, UV-B exposure rapidly induces dimer dissociation, producing activated monomers that interact with COP1 to initiate downstream responses, as described previously (Fig. 6b; Jenkins, 2014, 2017; Yin & Ulm, 2017). RUP2 accumulates and is proposed to displace COP1 to establish the photo-equilibrium (Fig. 6c). Continued exposure will lead to the acclimated state (Fig. 6d), where binding to COP1 is at a minimal level, sufficient to maintain a low level of gene expression, and binding to RUP2 is sufficient to maintain the dimer/monomer photo-equilibrium.

When UV-B-acclimated plants are transferred to a higher UV-B fluence rate both dimer and monomer photoreception will increase and both are likely to contribute to monomer activation, resulting in a stimulation of COP1 binding and hence an increase in target gene expression (Fig. 6e). However, RUP2 binding also increases to stimulate re-dimerisation of monomers to maintain the steady-state monomer abundance at *c.* 30% of total UVR8. The UVR8 population becomes more dynamic as the dimer/monomer cycling rate increases; elevated UV-B will increase the number of monomers binding COP1 and the number binding RUP2. These changes in interaction occur without any change in abundance of COP1 or RUP2 (Figs 3, 5).

Although elevated UV-B increases the proportion of monomers associated with COP1 and RUP2, it is not possible to estimate accurately the fraction of the total monomer population that is bound/unbound to these proteins. Some monomers are likely to be associated with other proteins, including WRKY36, BIM1 and BES1, which also interact with the C27 region (Liang *et al.*, 2018; Yang *et al.*, 2018). Further research is needed to develop a quantitative dynamic model of UVR8 action that takes into account these multiple interactions and to understand the factors that influence differential binding under particular environmental conditions.

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Author contributions

GIJ designed the research. XL and WL performed the experiments and analysed the data together with GIJ. H-QY provided reagents and technical guidance to WL. GIJ wrote the manuscript with input from all the authors. XL and WL contributed equally to this work.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Level of GFP-UVR8 expression.

Fig. S2 Additional representative blots.

Fig. S3 Production of anti-RUP antibodies.

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